

The Action of Light on Chlorophyll.

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(Received February 6,—Read March 5, 1914.)

The chemical changes brought about by light in the green leaf leading to the production of sugars and starch from carbon dioxide and water are still far from being clearly understood.

To what extent the chlorophyll takes part in this process, whether it simply performs the function of bringing the rays of light into contact with the carbon dioxide and water in such a way as to enable them to effect a synthesis of these two compounds, or whether the chlorophyll itself initiates these changes by its own chemical decomposition, are problems still unsolved.

It is a well-known fact that solutions of chlorophyll in the presence of oxygen become decolorised by light, and Pringsheim showed that the chlorophyll in a living leaf becomes rapidly blanched when submitted to the action of an intense light focused through a lens.*

The earliest observations on the destructive effect of light on chlorophyll appear to be those of Sir John Herschel, who in a series of papers† published more than 60 years ago described many interesting experiments on the action of the rays of the solar spectrum on the vegetable colours expressed from the petals and leaves of plants. From these experiments he concludes that (1) the action of light destroys colour, either totally, or leaving a residual tint on which it has no further or much slower action; (2) the action of the spectrum is confined, or nearly so, to the visible rays, as distinguished from the ultra-violet and ultra-red rays, which are ineffective; and (3) the rays effective in destroying a given tint are, in a great many cases, complementary to the tint destroyed. He pointed out that the green colouring matter expressed from leaves and spread on paper shows, as in the elder, a maximum of action, as indicated by the destruction of colour, in the red rays, from which the action falls off rapidly with a slight intermediate maximum in the region of the yellow, then falls off again, and about the termination of the green again increases, reaching another maximum in the blue violet, after which it falls off again, gradually, and ceases to be traceable as the termination of the violet is reached. He points out that "photographic pictures may be taken readily on such papers, half an hour in good sun sufficing; but the glairy nature of the juices prevents their being evenly tinted, and spoils their beauty." He did

* 'Pringsheim's Jahrb.,' 1881 and 1882.

† 'Phil. Trans.,' 1840, 1842; 'Phil. Mag.,' 1843.

not experiment with chlorophyll in a state of purity, owing to the nicety required in its preparation.

There is evidence to show that, under the influence of light, the chlorophyll in a living cell is constantly being destroyed, but that under normal conditions the leaves remain green, the chlorophyll being reconstructed as fast as it is destroyed. Thus when leaves are exposed to a stronger light than usual, they become paler in colour, probably owing to the fact that under these conditions the chlorophyll is destroyed at a more rapid rate than it is reconstructed. This is frequently observed in the leaves of shade plants when exposed to bright sunlight, and is also observed in Algæ such as *Spirogyra* which accumulate near the surface of water in the intense light of the sun during the summer months.

Thus Ewart* states that "when green leaves are exposed to sunlight, the decomposition of the chlorophyll goes on more rapidly than its production, though the amount of chlorophyll decomposed is insufficient to cause a change in the coloration visible to the eye."

Stahl came to the conclusion† that the exposure of leaves to direct sunlight for several hours gave no indication of the decomposition of chlorophyll.

Keeble showed, however, that leaves exposed to bright sunlight gave a weaker solution of chlorophyll in alcohol than similar leaves kept in the shade.‡ Many other experiments support this view, notably those of Ewart§ conducted on plants both in temperate and in tropical regions. Lubimenko|| has also shown that the quantity of chlorophyll in a leaf varies with the intensity of the light.

It is usually assumed that the decomposition of chlorophyll is bound up in some way with the photo-synthesis of CO₂ and water, but as it is usually considered to be more or less indirectly one of the results of photo-synthesis, a sort of by-product as it were, very little attention has, so far as I know, been paid to the products of its photo-decomposition.

Timiriazeff pointed out¶ that chlorophyll is a true optical sensitiser in that it absorbs radiant energy, and is at the same time an absorbent of one or more of the products by which the bleaching is then brought about. The function of chlorophyll is to decompose carbon dioxide; the chlorophyll absorbs the

* 'Linn. Journ. Bot.,' vol. 31 (1895-97).

† 'Ann. du Jard. Bot. de Buitenzorg,' vol. 11 (1893).

‡ 'Ann. Bot.,' vol. 9 (1895).

§ 'Journ. Linn. Soc. Bot.,' vol. 31; 'Ann. Bot.,' vol. 11 (1897); 'Ann. Bot.,' vol. 12 (1898); see also references in Pfeffer's 'Physiology,' Eng. Ed., vol. 1, p. 334.

|| 'Ann. Sci. Nat. Bot.,' 1908.

¶ 'Comptes Rendus,' 1885, and 'Ann. Sci. Nat. Bot.,' 1885; see also 'Roy. Soc. Proc.,' 1903.

rays of greatest energy and transmits this energy to the molecules of carbon dioxide.

Again, according to the hypothesis of Usher and Priestley,* the photo-synthesis of carbon dioxide and water is accompanied by the formation of hydrogen peroxide, and it is this latter compound that brings about the bleaching of the chlorophyll. From what we know of photo-chemical activity in other organic compounds, it would, however, not be unlikely that the rays of light absorbed by the chlorophyll may bring about a chemical change in it which is itself sufficient to initiate the series of chemical reactions resulting in the formation of sugar and starch. Thus Hoppe-Seyler,† quoted by Loeb,‡ “expressed the idea that chlorophyll undergoes first a combination with H_2CO_3 which, under the influence of light, falls apart in such a way as to yield chlorophyll (or the catalyser contained therein), O_2 and a third product, the latter being sugar or a substance from which sugar may be formed.” “It is obvious,” says Loeb, “that Hoppe-Seyler’s idea represents that conception of the action of the catalyser which is more and more supported by the facts.”

Hansen§ suggests that the chlorophyll is capable of forming an unstable compound with carbon dioxide, and that it is then passed on to the plasma of the chlorophyll grain to be converted into carbohydrate. Sir W. N. Hartley,|| in discussing this, says that it is, however, much more probable on chemical grounds that the compound of chlorophyll with carbon dioxide is entirely decomposed, first by the elimination of oxygen, and, secondly, by the elimination of water, so that there are successively formed compounds of chlorophyll (1) with carbon dioxide; (2) with formic aldehyde; (3) with glucose; and, finally, starch, completely formed, is split off the molecule.

The Bleaching of Chlorophyll in Light.

Crude chlorophyll was obtained in the ordinary way by boiling leaves of grass or other plants in water and then extracting with alcohol. Methylated spirit may be used for this purpose, but it is more satisfactory to use absolute alcohol. In order to obtain the chlorophyll in as pure a state as possible, the strong alcoholic solution was first filtered, then evaporated to dryness, and dissolved in petroleum ether. For many experiments ordinary ether will serve, but for general use petroleum ether is to be preferred. Paper tinged with chlorophyll, either in alcoholic solution or in petroleum ether solution, was used, and also films of chlorophyll made by the evapora-

* ‘Roy. Soc. Proc.’ vol. 77.

† ‘Physiologische Chemie,’ p. 139, Theil I (1877).

‡ ‘Dynamics of Living Matter.’

§ ‘Bied. Centr.’ 1888, see ‘Chem. Soc. Journ.’ Abstracts, 1888.

|| ‘Chem. Soc. Journ.’ 1891.

tion of the chlorophyll solution on glass plates and in glass tubes and flasks.

The bleaching of chlorophyll can be conveniently demonstrated by exposing the half of a strip of paper tinged with chlorophyll to the light, the other half being kept in the dark. In sunlight the bleaching takes place very rapidly, but very slowly in diffused light.

The action of the different rays of the spectrum can be shown by exposing a piece of paper tinged with chlorophyll or a glass plate covered with a layer of chlorophyll to a sunlight spectrum, and it will be seen that the bleaching takes place as described in Herschell's experiments and more recently by Reinke* and by Dangeard† in those parts of the spectrum where the light is absorbed. A convenient method of showing the different effects of the principal parts of the spectrum is to make use of filters through which definite wave-lengths are transmitted. The Wratten and Wainwright filters are suitable for this purpose, and the action of light is much more rapid than with the pure spectrum. The disadvantage of filters is that the different colours absorb varying proportions of the light which they are supposed to transmit. Thus whilst a red filter may transmit 78 per cent. of the light, a blue filter may transmit only 16 per cent. of it.

The tricolour set of filters supplied by Messrs. Wratten and Wainwright divide the visible spectrum into three nearly equal parts—red, green, and blue—with some slight overlapping, but as this green allows rather more of the yellow and blue ends of the spectrum to pass than is desirable, it is better to add to the green another one which limits its range. The different parts of the spectrum transmitted through the three filters which I have used are as follows:—

Red—Standard tricolour filter, λ 710–590.

Green—Standard tricolour plus green (two filters), about λ 550–480.

Blue—Standard tricolour filter, λ 510–400.

The bleaching of chlorophyll takes place very rapidly through the red filter, much more slowly through the green and blue filters. If, however, the light is allowed to act for a longer time through the blue and green filters, the bleaching then becomes as pronounced through the blue as through the red filter. Thus in bright sunlight it takes approximately 8–10 times as long to bleach chlorophyll paper through the blue filter as through the red. This seems to indicate that the different effects of the red and blue ends of the spectrum are proportional to (1) the absorption of light,

* 'Bot. Zeit.', 1885.

† 'Le Botaniste,' 1912 and 1913.

and (2) the energy coefficient of the different parts of the spectrum in which the absorption bands appear. Kniep and Minder* have pointed out that the effects produced in photo-synthesis are approximately proportional to the relative energy absorbed.

The Photo-decomposition Products of Chlorophyll.

The following experiments show that in the decomposition of chlorophyll by light two substances are produced, one giving the reactions of an aldehyde and the other an oxidising substance giving reactions with potassium iodide, by which the iodine is set free:—

Experiment 1: A piece of paper, tinged with alcoholic solution of chlorophyll, was arranged so that one-half of it was exposed to a good light, the other being kept dark. The half exposed to the light became bleached, and when placed in Schiff's solution the exposed portion developed a beautiful pink, the unexposed half remaining green with no pink coloration.

Experiment 2: If a piece of chlorophyll paper exposed to light as in Experiment 1 is placed in a solution of potassium iodide, the half exposed to the light becomes reddish-blue in colour, due to the liberation of the iodine, which acts upon the starch contained in the paper. The reddish-blue colour is probably due to the action of iodine upon starch in the presence of an excess of potassium iodide, for when the paper is washed in water the reddish-blue colour disappears and is replaced by the ordinary blue starch coloration.

Similar reactions to those described in these two experiments were found to take place when the paper itself was exposed to light without the chlorophyll, but the coloration was not so strong in either case.

Experiment 3: Two pieces of common note paper, similar to that used in Experiments 1 and 2, were exposed to light in the same way. One was placed in Schiff's solution. The exposed half became distinctly pink. The other was placed in potassium iodide and the exposed half became light brown. This seemed to indicate that the coloration in both cases was due to the paper and not to the chlorophyll.

Various kinds of paper were then experimented with, and it was found that in all cases a reaction both with Schiff's solution and with potassium iodide occurred, but that in the case of good superfine note paper the reactions were very slight. Accordingly, in all subsequent experiments with chlorophyll-tinged paper, a superfine note paper was used.

Experiment 4: A strip of W. H. Smith and Son's superfine cream laid note paper was tinged with chlorophyll and exposed to light as in Experi-

* 'Zeit. Bot.,' 1909.

ments 1 and 2. This was then cut longitudinally into two, and the two strips were then placed in Schiff's solution and in potassium iodide solution respectively. In both cases a strong reaction was obtained in those portions exposed to light. On comparing the results with the same paper not tinged with chlorophyll it was found that the reaction both in Schiff's solution and in potassium iodide solution was very strong with the chlorophyll-tinged paper but slight and almost negligible with the plain paper. Prolonged exposure of the plain paper to light gives a stronger reaction, but in no case as strong as the chlorophyll-tinged paper.

It was important to determine whether the solution of chlorophyll itself is able to give the reaction, apart from the paper. As alcohol gives a strong reaction with Schiff's solution it was necessary to dissolve the chlorophyll in some other solvent. For this purpose petroleum ether is suitable, as it does not give any reaction with Schiff's solution or with potassium iodide solution either in the dark or in the light.

Experiment 5: Four small test-tubes were partly filled with a solution of chlorophyll in petroleum ether and tightly corked. Two were exposed to the light and two kept in the dark. When those exposed to light were considerably decolorised, a small quantity of Schiff's solution was added to one and a small quantity of potassium iodide plus starch solution was added to the other. These solutions did not mix with the petroleum ether, but on shaking up the test-tubes the Schiff's solution became bright pink, the potassium iodide and starch solution became bluish-brown. The petroleum ether solutions which had been kept in the dark were treated in the same way with Schiff's solution and potassium iodide starch solution respectively, and in neither case was any reaction observed.

These experiments show clearly that the decomposition of chlorophyll is accompanied by the formation of an aldehyde and of a substance capable of oxidising the potassium iodide and setting free the iodine. It is extremely interesting to find that the same reactions are obtained with some kinds of paper when exposed to light. This is probably due to the decomposition of a substance in the paper the nature of which is unknown.

The same results are obtained when films of chlorophyll on glass are exposed to the light, and it can be further shown that the oxidising substance produced is a gas soluble in water.

Experiment 6: About 5 c.c. of a strong petroleum ether solution is carefully evaporated in a 50 c.c. flask so as to leave a thin film of chlorophyll on the sides and bottom of the flask. The ether should be completely evaporated, and a stream of air forced through the flask to remove all traces of the ether. The neck of the flask should be surrounded with black paper. A few drops

of distilled water are placed in the flask. A strip of potassium iodide paper about two inches long is then attached to a cork, and the flask is corked up so as to allow the strip of paper to hang down in the neck of the flask. Another flask should be fitted up in precisely the same way but without chlorophyll. Both flasks should now be exposed to the sunlight. The bleaching of the chlorophyll takes place very rapidly. The strip of potassium iodide starch paper becomes purplish blue in the chlorophyll flask, showing that iodine has been liberated, but remains quite unchanged in the control flask. The strip of potassium iodide starch paper is now removed and a few more drops of distilled water are placed in the flask, which is then corked and the contents well shaken up. The water in the flask is then poured into two tubes. To one of these a few drops of Schiff's solution is added and a pink coloration soon develops, showing the presence of an aldehyde. To the second tube a few drops of a 10-per-cent. solution of potassium iodide is added, and then on the addition of a freshly made starch solution, a blue or reddish-blue coloration is obtained, indicating the presence of an oxidising agent capable of setting free the iodine in the potassium iodide.

If the bleaching has been continued long enough, the sides of the flask are now covered with a thin white layer of a substance which should be well washed to get rid of the remnants of the soluble aldehyde, and it will then be found that this white substance is insoluble in either hot or cold water. If, however, the bleaching is prolonged for a considerable time a much smaller amount of the insoluble white substance remains.

If we expose the chlorophyll paper behind coloured filters, we find that both the aldehyde reaction and the potassium iodide reaction are much stronger in the red than in the blue and weakest in the green. If, however, the exposure behind the green and blue filters is prolonged to about 8 or 10 times that of the red, the reaction in the blue becomes as strong as in the red.

The reaction for aldehyde is therefore proportional to the bleaching effect, and is approximately proportional therefore to the synthetic activity in the different parts of the spectrum.

The longer the light is allowed to act, the more completely does the chlorophyll become bleached, with a corresponding increase in the aldehyde reaction. In the case of the potassium iodide reaction, however, the converse is the case. When chlorophyll films, either on paper or on glass, are submitted to the prolonged action of light, the reaction with potassium iodide is much weakened, and may be completely absent. The explanation of this is probably that the oxidising substance is a volatile gaseous product, which

tends to disappear as soon as it is formed, whilst the aldehyde is a solid which remains in the paper or in the film left on the glass. But it is not impossible that the oxidising substance may be of service in connection with the chemical reactions that take place in the chlorophyll, and may become used up in this way.

Instead of the extract of chlorophyll we may use dried leaves, or the chlorophyll expressed from living leaves and spread upon paper. We may also use layers of *Euglena viridis*, algæ and other green organisms spread over the surface of paper. In all these cases we can get by appropriate treatment, after exposure to light, both the aldehyde and potassium iodide reactions.

We can also show that both these reactions take place actually inside a leaf when the chlorophyll is sufficiently bleached. Thus if sunlight is condensed by means of a lens upon a living *Oxalis* leaf which contains abundance of starch, the chlorophyll in a small area of the leaf is bleached. If the leaf is now treated with Schiff's solution we get a strong aldehyde reaction in the bleached part; if treated with potassium iodide solution the starch grains in and around the bleached area become coloured blue. The last experiment is not an easy one to perform as it is very difficult to hit just the right moment to stop the bleaching in order to get the potassium iodide reaction.

Is Formaldehyde produced by the Photo-decomposition of Chlorophyll?

The observations of Pollacci,* Usher and Priestley,† Harvey Gibson,‡ and Schryver§ all show that formaldehyde is produced when chlorophyll is exposed to sunlight in the presence of carbon dioxide but not in its absence, or possibly in minute quantities only. It is therefore important to determine whether the aldehyde produced in my experiments is composed of formaldehyde or whether it contains formaldehyde. The test used by Harvey Gibson gives a very pronounced reaction even when formaldehyde is present in quite small quantities. I have obtained a reliable reaction with 1/1,000,000, and a very pronounced reaction with 1/100,000. The test is carried out as follows:—About 3 c.c. of pure concentrated sulphuric acid are placed at the bottom of a small test-tube; a few drops of a 5-per-cent. solution of gallic acid in absolute alcohol are poured gently on to the surface of the sulphuric acid and the liquid to be tested is then added; if formaldehyde is present, a beautiful

* 'Inst. Bot. d. R. Univ. di Pavia,' 1902; see 'L'Année Biologique,' 1903.

† 'Roy. Soc. Proc.,' 1906.

‡ 'Ann. Bot.,' 1907.

§ 'Roy. Soc. Proc.,' 1909.

blue-green ring appears between the upper and lower liquids. Unfortunately this test is not reliable, as dilute solutions of sugar and starch and various other substances bring about the formation of a green or blue-green ring. This may be due to the fact that the sulphuric acid decomposes such substances as starch and sugar, and that a transitory product of this decomposition may be formaldehyde. Consequently, although the reaction is extremely useful for purposes of preliminary test, it cannot be relied upon to prove the presence of formaldehyde.

Rimini's test, as modified by Schryver, is extremely sensitive to formaldehyde and will easily detect $1/1,000,000$. Here, however, the presence of various substances in the crude chlorophyll seems to interfere with the reaction, as shown by Schryver, and I have not been able to satisfy myself that the colour reaction given by this test with solutions of bleached chlorophyll is due to formaldehyde. Colour reactions are obtained which seem to indicate that formaldehyde is present in films exposed to light both in the presence and in the absence of carbon dioxide, but the reaction varies considerably with certain limits. Sometimes a dirty orange colour is produced, which is nothing like so distinct as the colour obtained with $1/1,000,000$ of formaldehyde, sometimes a deeper coloration which more nearly resembles the formaldehyde coloration, but is more of an orange red or deep orange than the bright, clear red of the formaldehyde reaction. In any case, none of my experiments shows more than a very small quantity of formaldehyde in this way, although the reaction given by Schiff's test in all cases indicated a much larger amount of aldehyde. For example, a solution of bleached chlorophyll showed a reaction for aldehyde with Schiff's solution equal to more than $1/25,000$, but on testing the same solution with Rimini's test, the result showed the presence of certainly not more than $1/1,000,000$ of formaldehyde. It appears to me from a large number of experiments that, although the aldehyde in the bleached chlorophyll may contain a small quantity of formaldehyde, the major part of it consists of some other aldehyde, the nature of which I have not been able to determine.

The Oxidising Compound of Chlorophyll.

The gaseous oxidising substance formed on exposure of chlorophyll to light is soluble in water. The experiments of Usher and Priestley suggested the possibility that it might be hydrogen peroxide. A solution was prepared by exposing a film of chlorophyll on water to the light, which gave a strong reaction with potassium iodide and starch, the iodine being liberated at once and colouring the starch blue. Various well-known tests for hydrogen

peroxide were then tried, but all gave a negative result. The following are some of the results obtained :—

Dilute solution of chromic acid with sulphuric acid. This gives a distinct blue coloration with $1/1,000,000$ of hydrogen peroxide, but no reaction with the chlorophyll solution.

One of the most delicate tests for hydrogen peroxide appears to be that given by Roscoe and Schorlemmer in their text-book. When hydrogen peroxide is added to a solution of potassium iodide and ferrous sulphate, iodine is set free. Other oxidising agents have the power of liberating iodine from potassium iodide, but not in the presence of ferrous sulphate. I have obtained a distinct reaction with $1/50,000$ of hydrogen peroxide and a reliable reaction with $1/1,000,000$. In the presence of ferrous sulphate the chlorophyll derivative gives no reaction, although the same solution gave a strong reaction with potassium iodide and starch alone.

Leuchter's test*: With this I obtained a very clear reaction with $1/500$ hydrogen peroxide, but no reaction with $1/50,000$. No reaction was obtained with a bleached chlorophyll solution.

Titanium dioxide in concentrated sulphuric acid gives an orange-red colour with $1/5000$ of hydrogen peroxide, and a distinct yellow colour with $1/50,000$; no coloration was given with the chlorophyll solution.

Experiments were also tried with a solution containing ferric chloride and potassium ferricyanide. This gives a precipitate of Prussian blue with solutions of hydrogen peroxide; solutions of the chlorophyll derivative only give a greenish yellow colour.

All these tests indicate, therefore, that the chlorophyll derivative is not hydrogen peroxide.

The experiment was then tried of exposing a film of chlorophyll in the dark to the action of a 20-per-cent. solution of hydrogen peroxide. If, as Usher and Priestley state, the decolorisation is brought about by hydrogen peroxide, we ought to get a very pronounced effect with so strong a solution. The experiment, however, showed that even after ten days' exposure to the hydrogen peroxide, the chlorophyll was far from completely bleached, and was still of a yellow or yellowish green colour. The experiment was tried many times in different ways, but always with the same result. The bleaching of chlorophyll in the light appears not to be due, therefore, to the action of hydrogen peroxide, and the most probable explanation seems to be that the light absorbed brings about a combination of the chlorophyll with oxygen resulting in the formation of an organic peroxide.

It is interesting to note that other colouring matters react to light in the

* 'Chem. Zeit.', 1911, see 'Chem. Soc. Journ.,' Abstracts, 1911.

same way with the formation of an oxidising substance capable of bringing about the liberation of iodine from potassium iodide. Thus, if strips of paper are soaked in solutions of the following dyes—methyl violet, methyl green, eosin, fuchsin, and fluorescein, and are then exposed to light and afterwards treated with a 10-per-cent. solution of potassium iodide, the iodine is liberated and the starch contained in the paper is coloured blue or reddish blue, a strong reaction being obtained in all cases. Cyanin, on the other hand, although readily bleached by the light, does not give this reaction.

Experiments made with narrow glass tubes lined with a thin layer of methyl violet and eosin show, on exposure to light, that, during the process of bleaching, oxygen is used up, but this is not the case with cyanin, which becomes completely bleached without any appreciable rise of water in the tube. In the case of methyl green and eosin, the absorption of oxygen does not take place as rapidly and is not so pronounced as in the case of chlorophyll.

The Photo-decomposition of Chlorophyll in a Brown Sea-weed—Laminaria.

In order to make experiments on the chlorophyll contained in the brown sea-weeds, I collected pieces of fresh fronds of laminaria on the sea-shore and brought them home wrapped in pieces of ordinary white paper. On removing the paper, I found a blue coloration here and there where the paper had been in close contact with the fronds. It was obviously the blue starch coloration due to iodine. I at first thought that it might be due to chlorine,* possibly contained in the paper, acting upon an iodine compound in the sea-weed and causing the liberation of iodine. A statement in Pfeffer's 'Physiology,' however, led me to suspect that the coloration might be due to free iodine given off by the laminaria itself. To test this, I took some fresh pieces of a frond of laminaria and placed them in a dilute starch solution free from chlorine. The solution became coloured blue, showing quite clearly the presence of free iodine. The colour disappeared again in a very short time, much more rapidly, so it appeared to me, than it would have done in a starch solution coloured by an ordinary solution of iodine. I accordingly tried the experiment again, and found on comparing it with a starch solution coloured with iodine to the same depth of colour, that the laminaria solution lost its colour several hours before the other. This indicated that the iodine was taken up again by the laminaria from the starch solution, and it occurred to me that this might be due to the slime which is secreted by the laminaria and which was found

* Chlorine is used in the bleaching of paper.

in large quantities in the solution. I therefore placed equal quantities of a light blue iodine-coloured starch solution in two test-tubes. To one of these I added distilled water; to the other an equal quantity of the slimy liquid obtained by soaking pieces of the frond of *laminaria* in water. The colour disappeared at once, on shaking up, in the tube containing the slime, but not in the tube to which distilled water only had been added. I then placed equal quantities of a very dilute iodine solution (iodine in potassium iodide) in two test-tubes. To one I added, as before, distilled water, to the other an equal quantity of the slimy liquid from *laminaria*. These were then shaken and left to stand for a short time. Equal quantities of a dilute starch solution were then added to each, with the result that the blue coloration appeared in the tube to which distilled water had been added, but no coloration at all in the tube containing the slime.

These experiments show, therefore, that iodine is absorbed by the *laminaria* slime, probably forming an additive compound with it, and it appeared probable that a much more satisfactory iodine reaction would be obtained with *laminaria* if the frond were first of all freed from slime by washing well in water. I obtained the reaction very readily on a bright spring morning on the sea-shore by placing pieces of the fresh frond free from slime in contact with starch paper. The reaction is, however, very unequal; all parts of a frond are capable of giving it, but not necessarily at the same time. The most vigorous reaction was obtained in the growing region of the frond, the swollen portion where the frond joins the stipe. The reaction appears to be associated with those layers of cells which contain the chlorophyll and the brown colouring matter. Sections of the stipe and of the thicker portions of the frond, when placed in contact with starch paper, showed a blue layer all round where the paper had been in contact with the peripheral chlorophyll-containing cells, and sections from the thinner portions of the frond also gave the same result.

Pieces of the frond kept in sea-water in the dark gave no reaction, or at times a slight one; a strong reaction was obtained when the fronds had been exposed to a good light. The presence of the slime, however, may prevent the reaction. So long as any of the brown colouring matter is present, the iodine reaction may be given, but it is entirely absent in those parts of the frond which have lost the brown colouring matter and show a green colour. Whether the iodine reaction is associated with the brown colouring matter I cannot say, but if one half of a piece of frond is placed for a short time in hot water to destroy the brown coloration, the iodine reaction is obtained only with the brown portions of the frond.

It is probable, therefore, though not quite certain, that the action of light

on the chlorophyll of laminaria brings about the production of an oxidising substance capable of effecting the decomposition of iodine compounds which may be contained in the chlorophyll cells of the plant, and that the iodine thus set free may either escape or be re-absorbed by the slime which occurs in such abundance in laminaria.

The Photo-decomposition of Chlorophyll takes place only in the Presence of Oxygen.

Three test-tubes were taken and lined with a film of chlorophyll by the careful evaporation of a petroleum ether solution of grass chlorophyll. One was placed with its open end in a strong solution of potassium hydrate and pyrogallol, to absorb the oxygen; the second was placed in a strong solution of potassium hydrate to absorb the carbon dioxide, and the third was placed in distilled water. All three were kept in the dark for 24 hours, and were then exposed to the light. The second and third bleached very rapidly. The first, which contained no free oxygen, remained unbleached even after an exposure of some months. At the time of writing, it has been exposed for four months in a south window and is still unbleached. The second and third gave strong reactions both for aldehyde and for the oxidising agent. The experiment shows quite clearly that the bleaching of chlorophyll is the result of oxidation brought about under the influence of light.

If this is correct, it occurred to me that we ought to be able to show that oxygen is absorbed in the process. To test this, I obtained four tubes 16 cm. long and 4 mm. in diameter; they were drawn out at one end to a point, which was then broken off, so as to leave an opening less than 1 mm. in diameter. Three of these were lined with chlorophyll from a petroleum ether solution. Care was taken to get rid of all traces of the ether by forcing a stream of air through the tubes for some time. I found a bicycle pump useful for the purpose. The fourth tube contained no chlorophyll and was simply used as a control. The three chlorophyll tubes and the empty tube (No. 4) were then placed with their open ends downwards in distilled water contained in two separate beakers. The water was at a sufficient depth to allow of its entry into the tubes to a height of 2 cm. This was done to allow of the expansion of the air in the tubes when they were exposed to the heat of the sun. The upper narrow ends of the tubes were then sealed with the bunsen flame. They were all placed in the dark for 12 hours. The next day they were all carefully measured, and it was found that the water was at the same height in each tube. One of the chlorophyll tubes and the control tube were then

exposed to sunlight. They were kept under careful observation to see that the expansion inside the tubes did not drive out any of the air. The chlorophyll in the chlorophyll tube soon began to bleach, and the level of the water began to rise and, in the course of a few hours, it reached a height of rather more than $1/5$ th of the tube, showing that part of the air, probably the oxygen, had been absorbed. The water in the control tube did not rise. The tubes were allowed to remain in the light until no further rise in the chlorophyll tube took place. At this stage the chlorophyll was not completely bleached, but as on prolonged exposure to sunlight no further bleaching took place, it seemed fair to conclude that all the oxygen had been used up. The tubes were now brought to the back of the room into diffused light and allowed to stand for some hours. Careful measurement of the chlorophyll tube showed that the level of water in the tube had risen to a height corresponding exactly to the percentage of oxygen likely to be present in the air enclosed in the tube when the experiment started. To test this, the control tube was placed in a strong solution of potassium hydrate and pyro. This gradually diffused into the water contained in the tube and then gradually absorbed the oxygen in the tube. In the course of 24 hours, the level of this solution had risen in the tube until it was stationary, and this was found to be almost exactly the same height as the level of the water in the chlorophyll tube. This showed pretty conclusively that in the bleaching of the chlorophyll the whole of the oxygen of the air had been used up. To show that no oxygen was left, however, the chlorophyll tube was itself placed in the potassium hydrate pyro solution; the tube was gently warmed until the water was nearly driven out, and then, on cooling, the pyro solution entered the tube and rose to exactly the same level as the level of the water previously contained in the tube, and remained at that level, thus showing that no oxygen had been left in the tube.

Experiments were then made with the other two tubes which had been kept in the dark; one was placed in a solution of potassium hydrate and pyro, the other was placed in the sunlight for some hours. On leaving them to stand for some hours at the back of the room to equalise the temperature, the level of the liquid in both was the same. Further experiments conducted more carefully with due attention to the corrections necessary for temperature and pressure showed quite conclusively that oxygen is absorbed when chlorophyll is bleached in the light, and that if sufficient chlorophyll is present, the whole of the oxygen in the air in contact with it is used up. It is possible, in fact, to make use of

chlorophyll instead of pyrogallol and potassium hydrate in making quantitative determinations of the amount of oxygen contained in the air.

The Presence of Carbon Dioxide is not Necessary for the Photo-decomposition of Chlorophyll.

The changes described in the last section are brought about just as rapidly and as completely in the absence of carbon dioxide as when it is present.

Thus, two flasks were prepared with films of chlorophyll from a petroleum ether solution of grass chlorophyll, as nearly as possible similar to each other. Into one 2 c.c. of distilled water were placed together with a short tube containing a stick of potassium hydrate to absorb carbon dioxide, and then tightly corked with a strip of potassium iodide starch paper in the neck of the flask. Into the second flask was placed 2 c.c. of water containing carbon dioxide from a sparklet apparatus, and this was then corked up, also with a strip of potassium iodide starch paper. Both were then exposed to sunlight, and it was found that the bleaching was equally rapid in both cases, that the potassium iodide starch paper was discoloured in both to the same extent, and that the aldehyde in both was similar in amount, so far as could be judged by the depth of colour produced with Schiff's solution. The experiment was tried several times under varying conditions, but the result was always the same, the presence or absence of carbon dioxide made no difference in the effects produced by the light.

But although carbon dioxide is not necessary for the photo-decomposition of chlorophyll, it is possible that, when present, it may be used up in some way corresponding to the photo-synthesis in the living plant. Experiments conducted with known quantities of carbon dioxide in contact with thin chlorophyll films in long narrow tubes, as used in previous experiments, showed, however, that, whether present in large or in small quantities, the carbon dioxide is apparently not used up in the bleaching of chlorophyll outside the plant. The bleaching takes place quite readily so long as oxygen is present, but the subsequent tests showed no diminution in the amount of carbon dioxide, so far as this could be measured with caustic potash.

The experiments are not conclusive, however. It is possible that a very small amount of carbon dioxide, too small to be measured quantitatively by the somewhat rough methods at my disposal, may be used up, but the fact that carbon dioxide is certainly not necessary for the bleaching of chlorophyll or the production of aldehyde, and that, so far as my experiments go, no appreciable amount of carbon dioxide is used up even when present in considerable quantities, would seem to indicate that, under the conditions of my experiments, carbon dioxide is not used up by the chlorophyll when

bleached in the light. It is important, however, that further experiments should be made in which the carbon dioxide determinations can be made more accurately.

The Photo-decomposition of the Green and Yellow Pigments of Chlorophyll.

The green and yellow pigments were obtained by shaking up an alcoholic solution of grass chlorophyll with benzene. The alcoholic solution of the yellow pigment was then evaporated to dryness and extracted with petroleum ether. The benzene solution of the green pigment was treated in the same way. Thin films of these two colouring matters were then exposed to light (*a*) in the absence and (*b*) in the presence of carbon dioxide. In both cases the yellow pigment bleached rapidly, and gave a very strong reaction both with Schiff's solution and with potassium iodide. The green pigment bleached much more slowly and did not give quite as strong a reaction with either Schiff's solution or potassium iodide. Similar results were obtained with strips of paper tinged with the green and yellow pigments respectively. Thus, paper tinged with yellow pigment from grass chlorophyll gave, after 40 minutes' exposure to diffuse sunlight in January, a strong reaction both with Schiff's solution and potassium iodide. The green pigment under the same conditions gave no reaction. Paper tinged with ordinary grass chlorophyll gave a slightly stronger reaction than the yellow pigment. At the end of two hours the green pigment gave a very slight reaction with Schiff's solution, but a strong reaction with potassium iodide, the yellow pigment a strong reaction in both cases.

The more rapid oxidation of the yellow pigment can also be seen by lining narrow glass tubes (*a*) with the yellow and (*b*) with the green pigment. These are then placed with their open ends downwards in water and exposed to bright sunlight. The water rises very rapidly in the tube with the yellow pigment, showing a rapid absorption of the oxygen, but more slowly in the tube with the green pigment. In both cases, however, the whole of the oxygen in the tube ultimately becomes used up, and the water rises to the same level in each.

The tubes were 39.4 cm. long. After exposure to light the water rose 8.6 cm. The height of the water in a control tube of the same length was 0.5 cm. Consequently on subtracting this both from 39.4 and 8.6, the ratio 8.1 to 38.9 gives 20.82 as the percentage of oxygen absorbed.

The Action of Oxidising Agents upon Chlorophyll.

As the decomposition of chlorophyll by light appears to be an oxidation process brought about by the oxygen of the air in the presence of light,

it occurred to me that similar effects might be brought about in the dark by the use of some of the ordinary agents such as hydrogen peroxide and permanganate of potash. I accordingly placed chlorophyll films, obtained by the evaporation of a petroleum ether solution of grass chlorophyll, in contact with a very dilute solution (pink) of permanganate of potash. These were allowed to act for six days and were then examined. In all cases the chlorophyll films showed considerable bleaching, and on carefully washing them with water to get rid of the oxidising agents and then bringing them into contact with Schiff's solution, a pronounced pink coloration was produced, showing the presence of an aldehyde. The aldehyde at first appeared in the film, but the colour soon became dissolved in the Schiff's solution, leaving a thin white layer in the glass. The powerful oxidising solution made by adding a few drops of sulphuric acid to a dilute solution of permanganate of potash acts very rapidly in bringing about the oxidation of chlorophyll and the production of an aldehyde. A film of chlorophyll placed in contact with the solution began to bleach at once, and in half an hour gave a very pronounced reaction with Schiff's solution.

The following experiments were also tried: A film of grass chlorophyll placed in the dark in contact with a 20-per-cent. solution of hydrogen peroxide for 16 days and then washed in water gave a strong reaction with Schiff's solution. The pink colour was first of all developed in the film, but soon became washed out in the solution, leaving a whitish layer on the glass.

A film of the yellow colouring matter of chlorophyll was treated in the same way, and gave a similar reaction with Schiff's solution.

A film of the green colouring matter of chlorophyll, treated in the same way, showed very slight decoloration or bleaching, and gave no reaction with Schiff's solution.

Similar results were obtained when strips of paper tinged with chlorophyll were used.

The bleaching of chlorophyll in the presence of hydrogen peroxide takes place much more rapidly in the light than in the dark. Thus, a film of grass chlorophyll was completely bleached in 12 hours in the light, but a similar film was hardly changed after ten days in hydrogen peroxide in the dark. In bright sunlight, complete bleaching was effected in three hours.

The film which had been bleached in the light gave a very strong reaction for aldehyde, and the oxidation of the film was so complete that only a trace of white film was left on the glass after the aldehyde had been dissolved out by the Schiff's solution.

The dilute sulphuric acid solution of permanganate of potash is a much

more powerful oxidising agent than hydrogen peroxide in its action upon chlorophyll. In the dark a very pronounced bleaching is obtained in half an hour, and nearly complete decolorisation is effected in about two hours, with a correspondingly strong reaction for aldehyde. In the light the bleaching takes place slightly more rapidly than in the dark.

The yellow colouring matter of chlorophyll bleaches very rapidly, the green colouring matter very slowly in permanganate of potash and sulphuric acid. Two films of equal size were prepared in two test-tubes, (1) of the yellow colouring matter, and (2) of the green colouring matter of grass chlorophyll, and equal quantities of the permanganate solution were poured into each with the following results:—

(1) Yellow colouring matter: In 50 seconds the permanganate solution was nearly colourless. At the end of two minutes it was poured off; the film was quite bleached and gave a strong reaction for aldehyde with Schiff's solution. The experiment was repeated with the yellow pigment from leaves of *Chrysanthemum parthenium* (Feverfew) with a similar result.

(2) Green colouring matter: At the end of one hour the permanganate solution was not quite colourless; the film still showed a yellow-green coloration, but was more strongly bleached in the thinner parts. In order to ensure more complete bleaching, fresh quantities of permanganate solution were added from time to time, but even at the end of 12 hours the bleaching was not complete. On testing with Schiff's solution, the film gave, however, a strong reaction for aldehyde.

None of the films bleached by oxidising agents, either in the dark or in the light, gave a reaction with potassium iodide.

Experiments with strips of paper tinged with (1) grass chlorophyll, (2) the yellow pigment, and (3) the green pigment from grass chlorophyll, gave similar results on treatment with the permanganate solution. (1) and (2) began to bleach at once, and at the end of half an hour gave a strong reaction for aldehyde; (3) showed a slight reaction only at the end of two hours.

From these experiments we may draw the extremely interesting conclusions that, so far as the production of an aldehyde is concerned, the oxidation of chlorophyll in the dark by means of solutions of hydrogen peroxide and permanganate of potash brings about a similar change to that which is effected when chlorophyll is acted upon by light in the presence of oxygen.

We have seen that the yellow colouring matter obtained both from grass chlorophyll and from the chlorophyll extracted from the leaves of *Chrysanthemum parthenium* bleaches very readily in the light, and also in

oxidising agents. This led me to suspect that the yellow colouring matter extracted from etiolated leaves—leaves in which only a yellow colouring matter had developed—would give the same results. The yellow-orange colouring matter extracted from etiolated rhubarb leaves was found, however, to bleach more slowly, both in light and in oxidising reagents, than ordinary chlorophyll obtained from grass, and very much more slowly than the yellow pigment from grass chlorophyll. Whether this has anything to do with the lack of photo-synthetic activity which Miss Irving* has found in chlorophyll not completely developed I cannot say, but, considered in the light of Miss Irving's observations, that the photo-synthetic activity of chlorophyll does not reach its full strength until the chlorophyll has been fully formed, the retardation of the photo-oxidation of the etiolin is of considerable interest.

It is, of course, possible that the yellow colouring matters from other plants may be found to behave differently in this respect, and too much stress must not be laid, therefore, upon the experiments just described. It is proposed to continue these observations.

The Action of Reducing Agents upon Oxidised Chlorophyll.

We have seen that the photo-oxidation of chlorophyll results in the production of an oxidising substance and of an aldehyde. Both are therefore oxidation products, and it was of some interest to ascertain the action of reducing agents upon them.

Three strips of paper coloured green by grass chlorophyll in a petroleum ether solution were exposed to the light until visibly bleached: 1 and 2 were then placed in a strong solution of phenylhydrazine, 3 was cut in two and one portion was treated with Schiff's solution, the other with a 10-per-cent. solution of potassium iodide. Both gave a strong reaction. After being kept in the phenylhydrazine solution for three hours, 1 was placed in Schiff's solution, 2 in potassium iodide solution, and in neither case was any reaction obtained.

Similar results were obtained with stannous chloride, and with a pyro-soda photographic developer.

Chlorophyll paper oxidised in the dark by the permanganate of potash solution, then treated for three hours with phenylhydrazine hydrochloride also gave no reaction either with Schiff's solution or potassium iodide.

The reducing agents do not bring back the green colour to the oxidised film, but the activity of both the products of chlorophyll photo-oxidation is destroyed.

* 'Ann. Bot.,' 1910.

Conclusion.

The experiments outlined in this paper indicate, so far as experiments conducted on dead chlorophyll extracts can be taken as an indication of what goes on in the living plant, that the bleaching of chlorophyll is not a result of the activities set up by photo-synthesis, as suggested by Usher and Priestley, but is the actual basis and starting point of the changes set up in the green leaf under the influence of light. In other words, the aldehyde produced under the conditions described in this paper is a product of the photo-decomposition or photo-oxidation of chlorophyll and is not a result of the direct photo-synthesis of carbon dioxide and water.

The aldehyde appears to be in fact purely a product of the photo-oxidation of chlorophyll. This modifies our conception of the changes which may possibly take place in the living plant. We know that carbon dioxide is necessary for the production of sugar and starch in the living cell. But if the sugar and starch are produced as the result of changes taking place in an aldehyde, and if the aldehyde is a direct product of the decomposition of chlorophyll, then we must conclude that the carbon dioxide before it can be used is built up independently into the chlorophyll molecule, and it is possible that the production of sugars and starch may be initiated by photo-oxidation of the chlorophyll rather than by the direct photo-synthesis of carbon dioxide and water.

Summary.

1. An account is given in this paper of some of the effects produced by light upon chlorophyll. When chlorophyll is exposed to the light at least two substances are formed, one of which is an aldehyde or mixture of aldehydes and the other an active chemical agent, capable of bringing about the liberation of iodine from potassium iodide.

2. These products of decomposition can be very easily demonstrated by means of strips of paper tinged with chlorophyll. When bleached in the light and placed in Schiff's solution, a deep pink colour is developed showing the presence of an aldehyde; but if placed in a 10-per-cent. solution of potassium iodide, a reddish blue coloration, which becomes blue on washing in water, is developed, showing the presence of an oxidising agent. The same products are obtained when films of chlorophyll on glass are bleached in the light.

3. The bleaching of chlorophyll is less at the blue end of the spectrum than at the red end, with a corresponding variation both in the aldehyde and potassium iodide reactions. But if the exposure to the blue light is prolonged to about eight or ten times that of the red light, the reactions are just as pronounced. The bleaching and the corresponding products of decomposition

are probably therefore proportional to the photo-synthetic activity of the chlorophyll in the different parts of the spectrum.

4. The presence of formaldehyde is not very clearly indicated in my experiments. Rimini's test, as modified by Schryver, gives indications of a trace of formaldehyde when chlorophyll is exposed to light both in the presence and in the absence of carbon dioxide, but I do not consider the results reliable, and in any case the reaction given is nothing like so strong as is indicated by Schiff's solution. The test used by Harvey Gibson is also very sensitive to formaldehyde, but is unreliable as it gives a pronounced reaction with solutions of sugar and starch and other substances. All that can be said at present is that in the photo-decomposition of chlorophyll a considerable quantity of aldehyde is formed, with possibly a small amount of formaldehyde.

5. The oxidising substance appears not to be hydrogen peroxide, but it may be an organic peroxide derivative of the chlorophyll.

6. The bleaching of chlorophyll *in situ* in dead green leaves, algæ, and other chlorophyll-containing organisms, gives the same products as the chlorophyll extracts outside the plant.

7. If a fresh green leaf of *Oxalis acetosella* is exposed to an intense light concentrated upon it by a lens, as in Pringsheim's experiments, the bleached chlorophyll gives an aldehyde reaction when placed in Schiff's solution. If the leaf contains abundance of starch, it may, after the action of the intense sunlight, be placed in a solution of potassium iodide, when the oxidising agent set free from the chlorophyll will liberate the iodine, and the starch grains will be coloured blue. The experiment is not an easy one to perform, as it is so very difficult to hit just the right moment to stop the bleaching, in order to get the potassium iodide reaction. See also the experiments on *Laminaria*.

8. The decomposition of chlorophyll with the production of aldehyde and peroxide takes place just as readily in the absence of carbon dioxide as when carbon dioxide is present. My experiments show that carbon dioxide is not used up in the process even when present in considerable quantities. Carbon dioxide is not necessary therefore to the production of the aldehyde.

9. The photo-decomposition of chlorophyll takes place only in the presence of oxygen. Oxygen is used up in the process. If sufficient chlorophyll is present, all the oxygen in the air in contact with the chlorophyll is absorbed. Chlorophyll may be used instead of caustic potash and pyrogallol in the analysis of air.

10. Chlorophyll is slowly oxidised in the dark by a solution of hydrogen peroxide. In the light the action is more rapid, but not more so than when light acts on chlorophyll in the presence of oxygen. A rapid oxidation of

the chlorophyll takes place in the dark in the presence of a dilute solution of permanganate of potash to which a few drops of sulphuric acid have been added. In both cases an aldehyde is produced which can be made evident by means of Schiff's solution.

11. If a strip of potassium iodide starch paper is exposed to light under coloured filters the paper turns reddish blue under the blue filter, showing the liberation of iodine, but not under the red filter. If, however, the iodised starch paper is first of all tinged with chlorophyll and then exposed to light under the same filters, a strong reaction takes place under the red filter.

A strip of bleached chlorophyll paper, placed in contact with a strip of damp iodised starch paper in the dark, is also capable of effecting the liberation of iodine, and the starch paper turns blue.

12. It is suggested in conclusion that the production of sugars and starch in the green leaf may be initiated by the photo-oxidation of chlorophyll and the subsequent polymerisation of the aldehyde thus formed, rather than by the direct photo-synthesis of carbon dioxide and water.

Intermittent Vision.

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(Received November 11,—Read December 11, 1913.)

[This paper is published in 'Proceedings,' Series A, vol. 89, No. 612.]

Studies in Brownian Movement. I.—On the Brownian Movement of the Spores of Bacteria.

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(Communicated by Principal E. H. Griffiths, F.R.S. Received November 19, 1913,—Read January 29, 1914.)

[This paper is published in 'Proceedings,' Series A, vol. 89, No. 614.]